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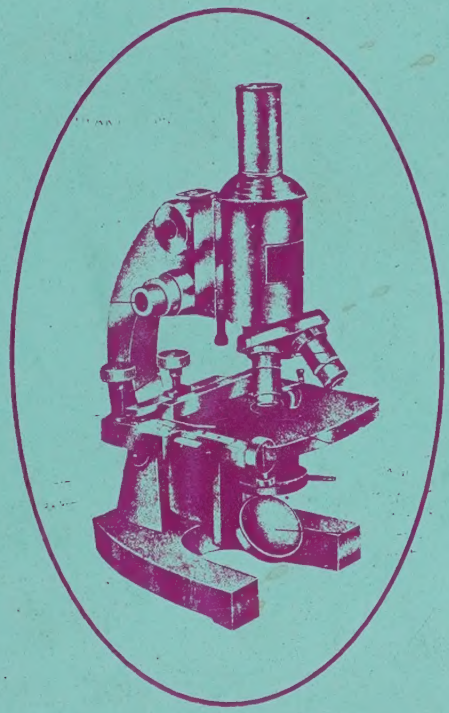
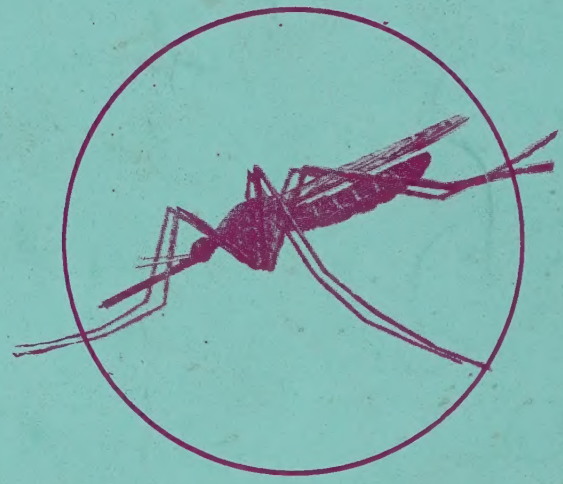
MANUAL FOR MALARIA MICROSCOPISTS





REGIONAL OFFICE FOR HEALTH AND FAMILY WELFARE
MINISTRY OF HEALTH & FAMILY WELFARE, GOVT. OF INDIA
BANGALORE

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PREFACE

In the National Malaria Eradication Programme, surveillance activities constitute the most important tool in arresting the transmission of the disease. After the blood smears are collected by the multi purpose workers, and also at the health institutions, examination is undertaken at the Primary Health Centers. This is the most important step. The laboratory technicians at the Primary Health Centers have to examine the blood smears meticulously, so that the efficiency of case diagnosis is maintained at a high level. The technicians are like the 'eye' of the programme.

To improve the working of the laboratory technicians, both induction and in-service orientation training are essential. This module is prepared for facilitating the training. It should also help them on a continuous basis even after the training programme, as it contains useful referral material.

This module is based on the "Instructions for malaria microscopists" - developed by the Directorate of National Malaria Eradication Programme in 1987.

Dr. K. Ravi Kumar
Chief Medical Officer

Dr. P.K. Shome
Senior Regional Director

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IMPORTANT INFORMATION ABOUT MALARIA FOR A LABORATORY TECHNICIAN

- **What is Malaria ?**

- Malaria is a fever caused by the presence of malaria parasites in human body.

- **What are the symptoms of malaria fever ?**

- The patient gets rigor followed by high fever daily or on alternate days.

A PERSON SUFFERING FROM MALARIA WILL ALWAYS GET FEVER
BUT
ALL FEVERS ARE NOT DUE TO MALARIA

- **How does malaria spread ?**

- Malaria parasite enters into the body of female anopheline mosquitoes whenever they bite a patient of malaria. It takes 10- 15 days for its development in mosquito before this disease can be transmitted to a healthy individual by the bite of this infected mosquito.

- **How many days after the bite of an infective mosquito will a human being get fever ?**

- That individual will suffer from fever 8 to 12 days after the infective bite of the mosquito.

- **Where does malaria parasite reside in the body of a human being ?**

- This parasite is found in the red blood cells of human being.

THEREFORE IT IS VERY IMPORTANT TO EXAMINE THE BLOOD SMEAR
OF A PATIENT SUFFERING FROM FEVER AS SOON AS POSSIBLE

- **Aims of prevention/control of Malaria:**

- Healthy individual should not get malaria infection.
  (Method - Mosquitoes should be killed by spraying houses with DDT)
- To give radical treatment to all patients of malaria in a community after confirmed diagnosis.

- **How to diagnose a case of Malaria ?**

- *This is done in two steps:*
- (A) *A blood smear* should be made *from every fever case* either by a surveillance worker/MPW or a passive agency and correct address recorded.
- (B) Every blood smear should be examined under the microscope for detection of malaria parasite.

A MALARIA PATIENT CAN ONLY BE DIAGNOSED BY CAREFUL EXAMINATION AND CORRECT IDENTIFICATION OF MALARIA PARASITE IN BLOOD SMEAR

- **What are the essential requirements for an accurate examination of a blood smear ?**

- The blood smear from a fever patient should be drawn/made properly.
- The staining of blood smear should be of high quality.
- The blood smear should be examined by a technician skilled in malaria microscopy and the result should be declared after examining the required number of microscopic fields.

- **What action is to be taken immediately after obtaining the result of blood examination ?**

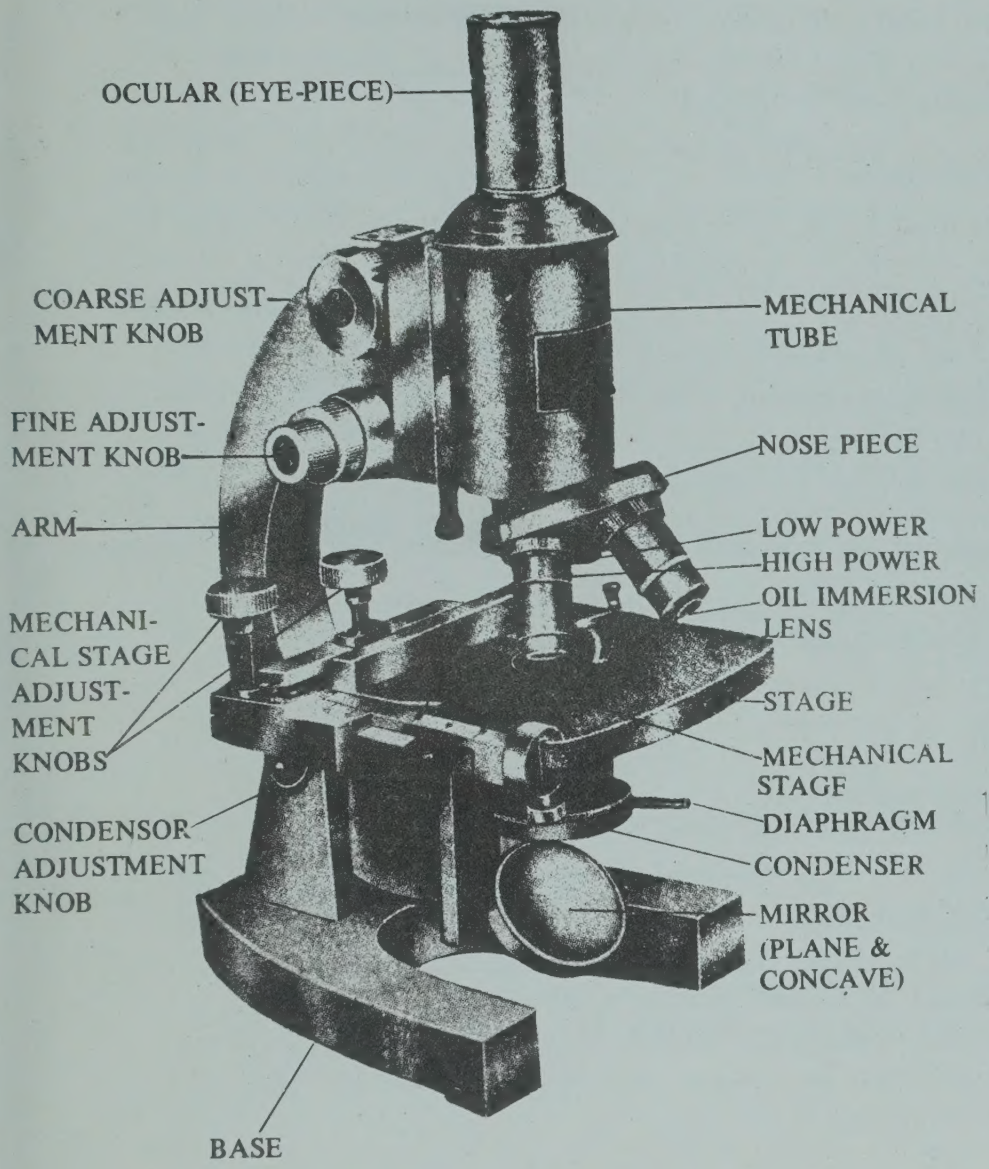
- Correct and complete address of the positive case should be entered in the form/registers.
- Correct species of the positive case should be mentioned in records.
- Medical Officer in-charge should send information to the concerned surveillance inspector for radical treatment.
- Complete information should be entered in the laboratory registers in a proper manner.

- **What action should be taken by the laboratory technician, if a large number of positive cases are being detected from a particular section or village during a fortnight ?**

- If such a situation arises, the Medical Officer in-charge, Malaria Inspector and Malaria Officer should be informed immediately.

IN THE EVENT OF A SUDDEN INCREASE OR DECREASE IN THE NUMBER OF BLOOD SMEARS COLLECTED OR POSITIVE CASES DURING A FORTNIGHT FROM A DDC/FTD OR A SECTION, LABORATORY TECHNICIAN SHOULD INFORM IMMEDIATELY THE M.O. IN-CHARGE OF PHC & DISTT. MALARIA OFFICER.

COMPOUND MICROSCOPE



LIST OF EQUIPMENT

1. Compound microscope.
2. 50 cc. glass beakers (at least-6) and watch glasses to cover them.
3. A staining tray - size (10" x 8" x 2")
4. An enamel tray for dehaemoglobinisation with glass rods (18" x 14" x 3").
5. A glass funnel and funnel stand.
6. Filter paper ordinary (non-analytical grade).
7. Buffer water salts in packets for making 2000 cc. of buffer water.
8. Two or three enamel basins of 18" diameter or enamel trays of size 18" x 14" x 3" for clearning of glass slides.
9. In PHCs with electricity, a flourescent tube light to be fixed on the laboratory table.
10. Laboratory table with drawers and side cupboards.
11. Stool - revolving.
12. Pigeonhole cabinet.
13. Two buckets of 15 or 20 litres and two mugs.
14. Rack for keeping records.
15. Two 2000 cc. bottles for storing buffer water.
16. Four 500 cc. bottles for storing JSB I and II.
17. Stationery for maintaining records.
18. MF-7, 8, 9 Registers.
19. Glass slides and equipment for taking blood smears.

METHOD FOR CLEANING NEW AND USED GLASS SLIDES

- **EQUIPMENT REQUIRED:**

- Enamel wash basin (Diameter = 18") or an enamel tray (18" x 14" x 3")
- Sufficient quantity of clear water
- Detergent solution or Surf/soap powder
- Glass slides (new or used)

- **METHOD:**

- Take 3-4 litres clean water in the basin.
- Add 3-4 cc of zedinol or 75 gm Surf powder and mix.
- Dip 400 glass slides in this solution.
- Wait for 12-24 hours. In the case of an urgency, use hot solution and then after 2-3 hours follow the procedure given below;
- Clean every slide with a cloth so that the existing blood smear on the slide is removed.
- Wash these glass slides twice with clean water and
- Dry them by wiping with a piece of clean dry cloth.
- Pack these dry slides in paper - 25 to 50 glass slides per packet.

PRECAUTIONS

- DIRT AND POWDER FROM NEW SLIDES AND THE PREVIOUS BLOOD SMEAR FROM OLD SLIDES SHOULD BE COMPLETELY REMOVED.
- THROW AWAY ALL BROKEN SLIDES AND ALSO THOSE WITH SCRATCHES.

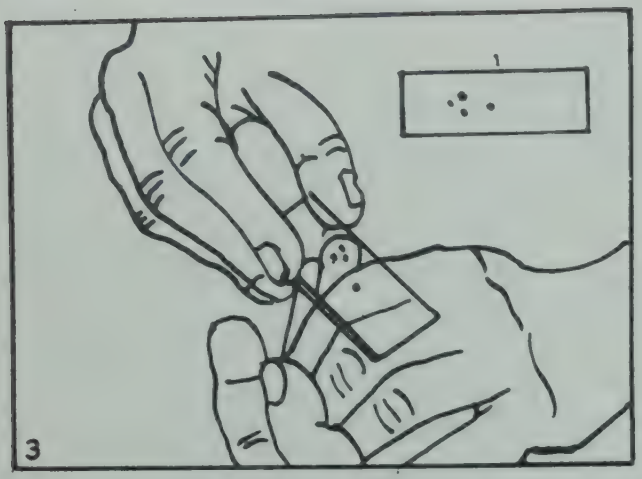
HOW TO PREPARE A THICK AND THIN BLOOD SMEAR FOR MALARIA MICROSCOPY

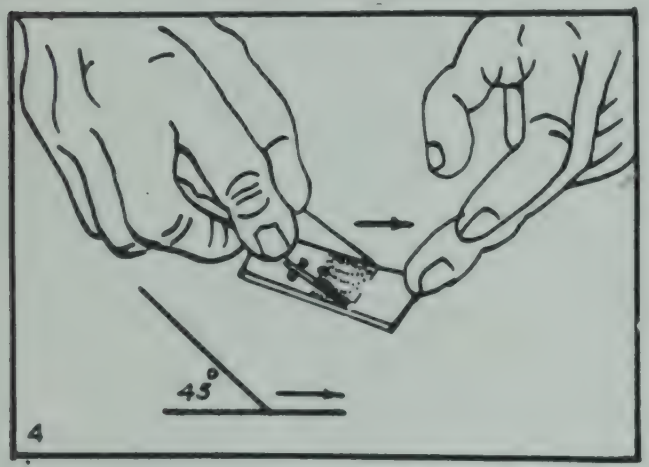
- **EQUIPMENT REQUIRED:**
- Clean glass slides
- Pricking needle (Hagedorn Triangular No. 12)
- Specimen tube with cork for fixing pricking needle
- Spirit or Savlon solution
- Small bottle with cork for keeping spirit or Savlon solution
- Cotton
- Clean handkerchief
- Slide box for 25 or 50 slides.
- Lead pencil
- Register and MF-2 forms
- Carbon paper
- Ball point pen
- Antimalarials for giving presumptive treatment.

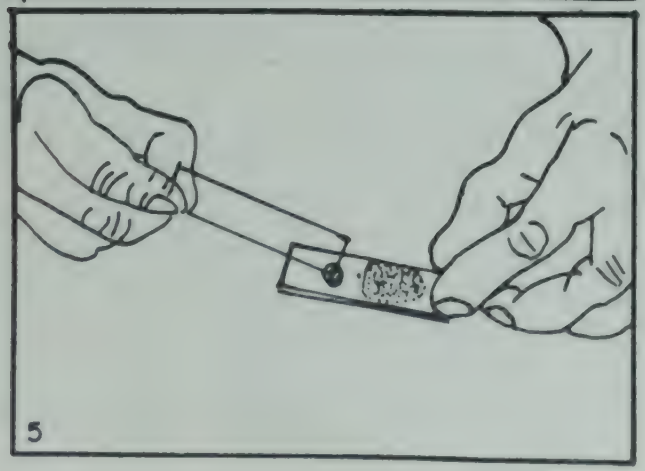
METHOD:

1. - Third finger of the left hand of the patient should be held with left hand between thumb and finger by blood slide collector at the first phalangeal joint.
   - Wipe finger tip with swab dipped in spirit or Savlon solution.
   - Allow the finger tip to dry. **(Figure-1)**

2. - Hold the pricking needle in right hand and prick the finger.
   - Allow blood drop to ooze out. **(Figure-2)**







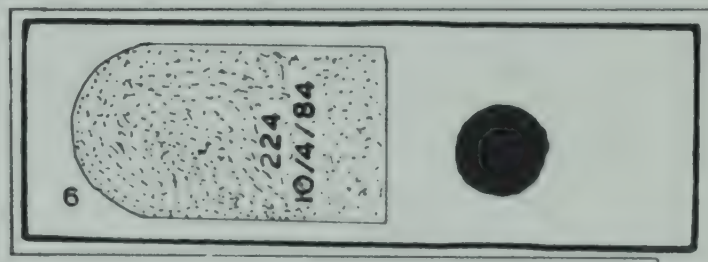
METHOD (Contd.)

3. - Take a clean slide.
   - Take 3 drops of the blood (sufficient blood) 1 cm. from the edge of the glass slide.
   - Take another drop of blood one cm from the first drop of blood.

(Figure - 3)

4. Take another clean slide with smooth edges and use it as a spreader.
   - Make thick and thin smears.
   - Allow it to dry.

(Figure - 4 and 5)



5. – Put the slide number on **thin smear** with lead pencil.

(Figure–6)

Quality of Thick Smear:

- It is 10 mm. away from the edge.
- It is round in shape with 10 mm. dia.
- Its thickness contains 10 layers of Red blood cells.
- 10-12 WBC should be visible in one oil immersion field of microscope.

PREPARATION OF J.S.B. STAINS

J.S.B.I.

INGREDIENTS

1. METHYLENE BLUE (MEDICINAL)	0.5 gm
2. SULPHURIC ACID (H_2SO_4) 1%	3. c.c.
3. POTASSIUM DICHROMATE ($K_2Cr_2O_7$)	0.5 gm.
4. DISODIUM HYDROGEN PHOSPHATE DIHYDRATE ($Na_2HPO_4.2H_2O$)	3.5 gm.
5. DISTILLED WATER	500 c.c.

J.S.B.II.

1. EOSIN YELLOW (WATER SOLUBLE)	1.0 gm.
2. DISTILLED WATER	500 c.c.

BUFFER WATER

1. WATER	1000 c.c.	2000 c.c.
2. DISODIUM HYDROGEN PHOSPHATE DIHYDRATE ($Na_2H\ PO_4.2H_2O$)	0.22 gm.	0.417 gm.
3. POTASSIUM ACID PHOSPHATE (KH_2PO_4)	0.74 gm.	0.752 gm.

EQUIPMENTS

1.	PHYSICAL BALANCE	1
2.	FLASK 1000 c.c.	1
3.	BEAKER 500 c.c.	1
4.	MEASURING CYLINDER	500 c.c. 1
		100 c.c. 1
		10 c.c. 1
5.	PIPETTE	5 c.c. 1
		c.c. 1
6.	FILTER PAPER ORDINARY (PACKET)	1
7.	TRIPOD STAND	1
8.	STAND AND CLAMP FOR HOLDING FLASK	1
9.	STOVE	1
10.	DISTILLED WATER APPARATUS	1
11.	REFLEX CONDENSER	1
12.	WIRE GAUZE	1

NOTE

1. Methylene Blue or Eosin should be manufactured by a reputed firm and all other chemicals should be analytical quality (of highest purity).

2. In J.S.B.I. quantity of $Na_2\ HPO_4$ will differ according to H_2O molecule: (Anhydrate 2.79 gm; $2H_2O$ - 3.5 gm.; $5H_2O$ - 4.56 gm.; $7H_2O$ - 5.26 gm.; $12H_2O$ - 7.04 gm.)

METHOD OF PREPARATION

J.S.B.I.

A. Measure 500 c.c. of water in a flask with measuring cylinder.
B. Add 0.5 gm. methylene blue in 500 c.c. of water, shake well to dissolve.
C. 3 c.c. sulphuric acid 1% should then be added in three parts (1 c.c. at a time) and solution stirred thoroughly.
D. 0.5 gm potassium dichromate is added and mixed by shaking till blue colour turns violet and precipitate forms.
E. Now 3.5 gm. disodium hydrogen phosphate dihydrate is added and solution is shaken till precipitate dissolves.

A GOOD SOLUTION IS PRECIPITATE FREE AND VIOLET IN COLOUR

F. Put this solution on low flame. Note time when boiling starts and boil slowly for 1 hour.

NOTE :

Boil the solution on low flame using condenser, otherwise more water will evaporate changing its concentration.

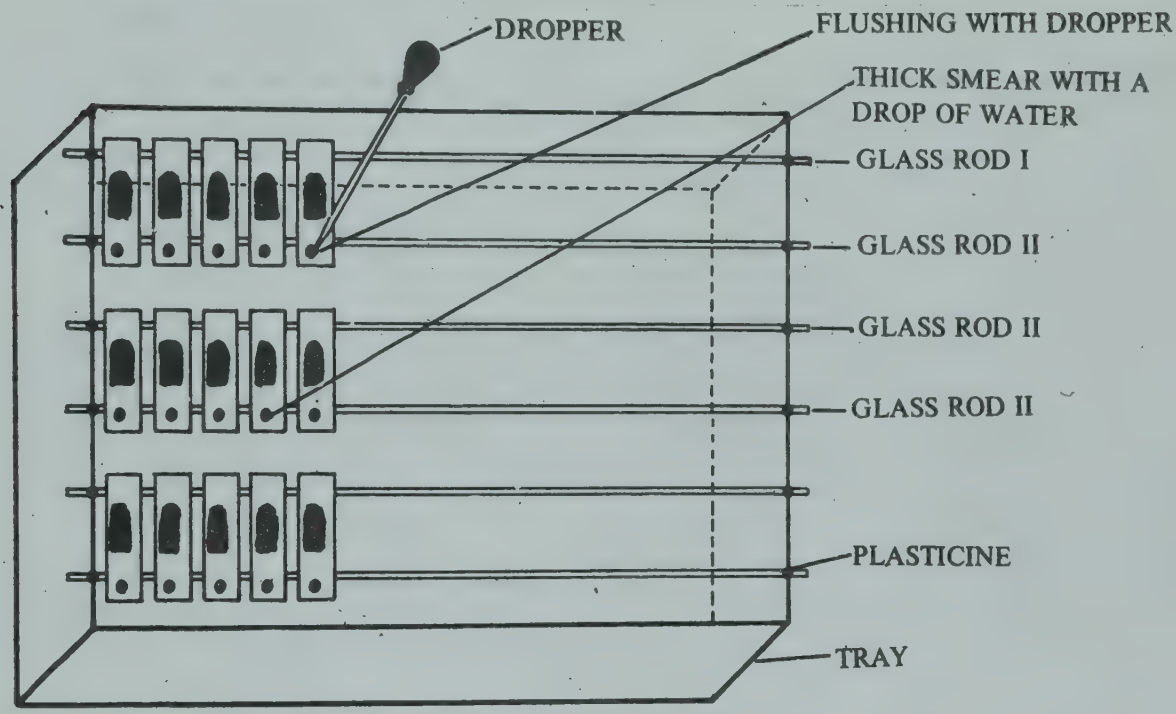
J.S.B.II

Dissolve 1 gm eosin in 500 c.c. of water. Allow to mature for 48 hours before use.

BUFFER WATER : - Take distilled water. If not available, well or tap water filtered through filter paper. Add specified quantity of buffer salt in 1000 or 2000 c.c. of water as required and indicated above. pH of the solution should be approximately 6.2 to 6.8.

A mature solution after it cools down should be violet in colour and deep blue with violet irridescence - If it is blue or green, it should be thrown away and a fresh stain may be made.

DEHAEMOGLOBINISATION OF BLOOD SMEARS



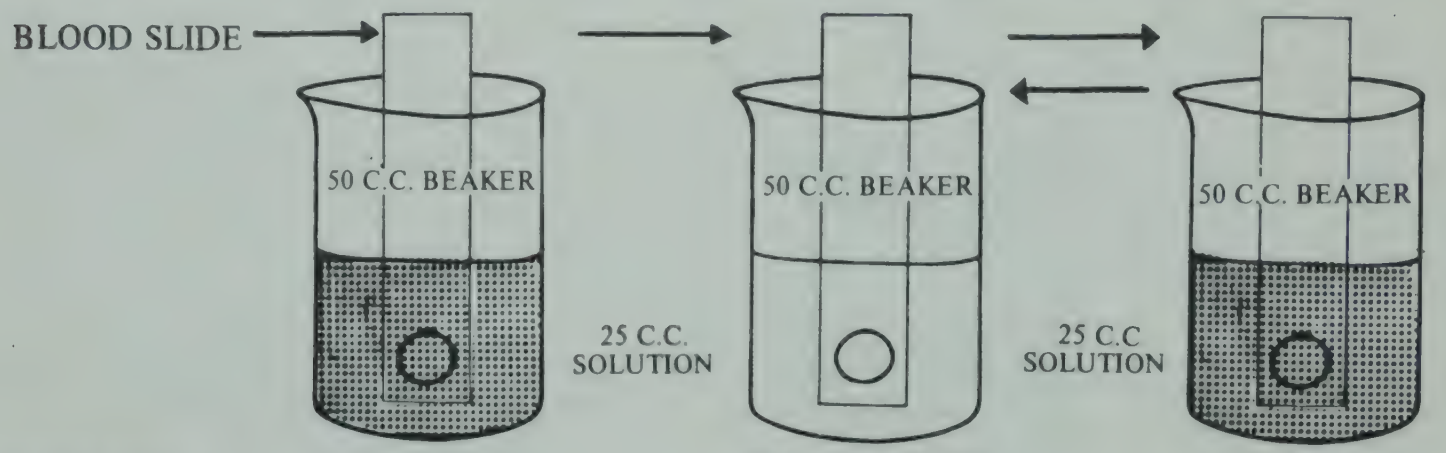
- Draw a line with a glass marking pencil between thick and thin smears.
- Arrange blood slides on glass rods as shown in the above diagram with thick smear towards second glass rod.
- Put a few drops of water on thick smear to cover it completely.
- Wait for 5-7 minutes.

PRECAUTION
USE CLEAN WATER ONLY. IT SHOULD NOT HAVE ANY SUSPENDED MATERIAL.
(IF TAP OR WELL WATER IS USED, IT SHOULD BE FILTERED THROUGH AN ORDINARY FILTER PAPER)

Flush the thick film by pouring water with the help of a dropper.

NOTE : 1. EVERY SECOND GLASS ROD SHOULD BE AT A SLIGHTLY LOWER LEVEL THAN THE FIRST GLASS ROD.
2. THICK FILM SHOULD BE STAINED AFTER DRYING COMPLETELY, OTHERWISE THE FILM WILL BE FLUSHED AWAY.

IMPORTANT INSTRUCTIONS FOR STAINING OF BLOOD SLIDES



ALL BLOOD SLIDES SHOULD BE DEHAEMOGLOBINISED AND STAINED WITHIN 48 HOURS OF THE RECEIPT IN THE LABORATORY

USE OF BUFFER WATER IS ESSENTIAL

To make buffer water use distilled water. If not available then well or tap water. Filter through filter paper. Add one packet of buffer salt to 1000 cc. of water. Packet of buffer salt should contain (1) Disodium Hydrogen Phosphate Dihydrate - ($Na_2 HPO_4 . 2H_2O$) - 0.22 gm. (2) Potassium Acid Phosphate - (KH_2PO_4) - 0.74 gm.

1. After dehaemoglobinisation, dip the thick smear in JSB II Stain (Two to three times).
2. Wash it by dipping in buffer water two to three times.
3. Then keep thick film dipped in JSB I stain for 40-60 seconds.
4. Wash thick film with buffer water.
5. Keep the blood smear for drying.

- **Precautions to be observed while staining blood smears:**
- Every blood slide should be completely dehaemoglobinised before staining.
- Put only 25 cc. of JSB I or II after filtration in a beaker of 50 cc. capacity.

- Used filter paper should be kept as record after signing it with date.
- Used stain solution should never be filtered again.
- Used stain solution should never be mixed with stock solution.
- Throw away the stain solution after staining 60-70 blood smears.
- If more blood smears are to be stained, take 25 cc. of fresh JSB I and II after filtration.
- Change buffer water whenever it gets light blue colour.

IF ON A PARTICULAR DAY A LARGE NUMBER OF BLOOD SLIDES ARE RECEIVED THEN STAINING SHOULD BE DONE IMMEDIATELY AND COMPARATIVELY LESS NUMBER OF BLOOD SMEARS SHOULD BE EXAMINED TO COMPENSATE FOR EXTRA TIME TAKEN FOR STAINING. THE NUMBER OF BLOOD SMEARS STAINED SHOULD BE ENTERED IN THE REQUIRED REGISTER EVERY DAY.

IT TAKES ABOUT ONE HOUR AND 15 MINUTES FOR DEHAEMOGLOBINISATION AND STAINING OF 60 BLOOD SMEARS

- Thin film should not be dipped in buffer water or stained while staining thick film.
- If thick film cannot be dehaemoglobinised due to fixation of film, the result should be given after examination of the thin smear.

THE THIN FILM SHOULD BE FIXED IN METHYL ALCOHOL (METHANOL) BEFORE STAINING.

IN A STAINED THICK SMEAR AGAINST UNIFORMLY BLUISH/PINK TRANSLUCENT BACKGROUND

(A) NO RBCs ARE SEEN, ONLY A FAINT CELL-OUT LINE MAY BE SOMETIMES VISIBLE.

(B) ONLY STAINED PLATELETS, WBCs AND PARASITES ARE SEEN.

SOME COMMON DIFFICULTIES FACED WHILE STAINING THICK FILM AND THE MEASURES TO REMOVE THEM

- **No blood smear is seen on the slide after staining.**

REASON: It may have been washed away;

(a) due to inadequate drying of blood smear or because of its excessive thickness;

(b) due to carelessness during dehaemoglobinisation or staining of slide.

REMEDY : To observe all precautions while staining.

- **Proper background is not obtained and thick blood film is full of artefacts.**

REASONS: (a) Use of dirty slide;

(b) pricking a dirty finger;

(c) change of getting bacterial growth increases if the slide is dried very slowly;

(d) settling of dirt on the slide while drying.

(e) appearance of thin coloured lines or small particles on the film due to fungus.

REMEDY: for (a) and (b) above

Use clean slide and clean the finger thoroughly with spirit.

(If spirit is not available Savlon is to be used).

USE OF CLEAN SLIDE IS MORE IMPORTANT FOR THICK FILM THAN THIN FILM

(c) While drying, slide should be kept covered in a slide box to protect it from dust.

(d) Film should be spread rapidly but smoothly, otherwise the desired results will not be achieved. Good results will be achieved only by making a film as quickly as possible.

FILM SHOULD BE SPREAD WITHIN 2-3 SECONDS

(e) Before dipping the slide in stain solution, a layer of scum present on the surface of stain solution should be removed.

- **In thick film a dark background**

REASONS

(a) Excessive thickness of film.

(b) Fixation of blood film which may be due to following reasons:

1. Delay in staining
2. Due to the effect of excessive heat or normal heat fixation when blood smears already collected are carried by workers during their daily rounds.
3. Due to contact of blood with spirit which was applied to clean the finger.
4. Due to vapours of spirit while fixing thin film.
5. Keeping the slide in stain solution for a longer time than prescribed and not washing it properly.

REMEDY:

(a) Blood smear should be stained as soon as possible.

(b) Spirit on finger should be dried completely before giving it a prick.

(c) After pricking, the first drop of blood should not be used for making blood film.

(d) Blood slide should be kept away from all harmful effects before dehaemoglobinization.

- **Deep red or blue background in the film.**

REASONS:

Excessive acidity or alkalinity of the solution.

REMEDY :

Monitor the pH of water and buffer and use a good quality stain.

- **If WBC and parasite appear faint in colour or with bluish green tinge.**

REASONS:

(a) Less time was spent in staining the slide.
(b) Stain was old.
(c) pH of buffer water was not correct.

REMEDY:

(a) Adequate time should be spent while staining blood smear.
(b) Watch for the quality of stain.
(c) Examine the pH of buffer water.

- **Parasite and WBC appear broken.**

REASON: Inadequate drying of blood film.

REMEDY: Blood film should be stained after at least half an hour of complete drying to get good results.

- **Appearance of artefacts in blood film.**

REASON: Use of old slide, laden with bacteria and dust, old stock of distilled water, buffer water or stain which might be contaminated with bacteria, fungus and other impurities.

REMEDY: All instructions should be followed carefully and only clean slide should be used.

PRECAUTIONS TO BE OBSERVED DURING BLOOD SMEAR EXAMINATIONS

Proper way of using a Microscope:

- **Always use both hands while carrying the microscope. Hold the arm of the microscope with one hand and give a support at the base with the other hand.**
- **Always clean the eye-piece and oil immersion lens before and after use with grease-free cotton cloth (not lint or silk cloth).**
- **While microscope is on the table but not in use;**
  - i) keep the condenser in low position;
  - ii) change the objective from oil immersion to low power;
  - iii) remove the blood slide from the stage; and
  - iv) cover the microscope with cloth or plastic cover.

SUGGESTIONS REGARDING EXAMINATION OF BLOOD SLIDE

- **If the number of blood slides is about 70, then all of them should be examined on the same day.**
- **If the receipt of slides is more than average, the following priority in examination should be observed.**
  - i) Blood slides from malaria clinic should be examined immediately.
  - ii) Blood slides from passive agencies.
  - iii) Blood slides from a project.
  - iv) Blood slides from those sections where a high incidence of *P. falciparum* was reported in the previous year.

IF THERE IS INCREASE IN A BACKLOG, INFORM YOUR MEDICAL OFFICER IN-CHARGE OF PHC AND MALARIA INSPECTOR OR MALARIA OFFICER

ONE TECHNICIAN SHOULD EXAMINE 60 to 70 BLOOD SMEARS IN A DAY.
AT LEAST 100 MICROSCOPIC FIELDS OF A THICK FILM SHOULD BE EXAMINED UNDER OIL IMMERSION LENS.

SOME USEFUL PARAMETERS REGARDING HUMAN BLOOD

- Blood is a red coloured fluid which flows in blood vessels of a human being. Its weight is approximately 1/20th or 5% of the total body weight. Normally, it is 4 to 6 litres.

- Blood is a mixture of solid particles and a liquid component.
  (a) Liquid component is called plasma.
  (b) Cells, salts, minerals and proteins constitute the soild particles.

- Cells are of three types:
  (a) Red blood cells;
  (b) White blood cells; and
  (c) Platelets.

- Red blood cells are shaped like biconcave discs with 7.2 micron diameter and 1 micron in thickness. They are red in colour and are normally 4 to 5 million in one cubic mm. of blood. The red colour of the RBC is due to presence of haemoglobin which is about 14 to 16 gm. in 100 cc. of blood.

- White blood cells are about 6 to 10 thousand in one cubic mm. of blood. They are of two types;
  (a) Agranulocytes; and
  (b) Granulocytes.

- Agranulocytes are also of two types;
  (a) Lymphocytes; and
  (b) Monocytes.

- Lymphocytes constitute 25-30% of all WBCs, are 6-10 microns in size and have one nucleus which is deep violet in colour, and approximately fills up the cell.

- **Monocytes constitute 2-5% of all WBCs, are 9 to 20 microns in size and have one nucleus which is kidney or horseshoe shaped and dark blue in colour.**

- **Granulocytes are of three types;**

  1. *Neutrophils* (Polymorphonuclear) - Nucleus has one to five lobes and is deep violet in colour. Granules do not take the colour. It is 10-12 microns in size and constitutes 60 to 70% of all WBCs.

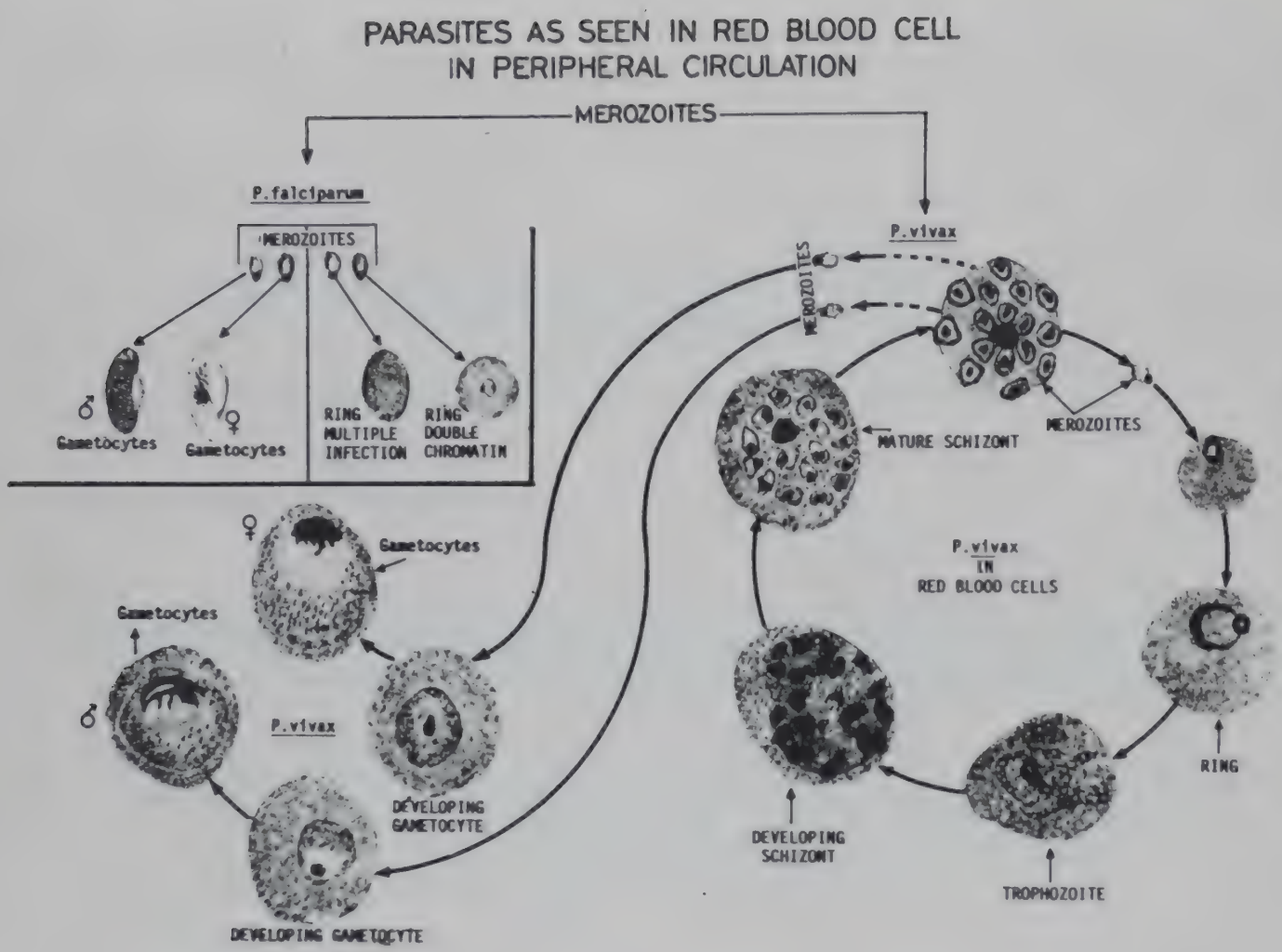
  2. *Eosinophils* - Nucleus is bilobed and kidney shaped. Granules are dark red in colour. It is 10-12 microns in size and constitutes 1-5% of all WBCs.

  3. *Basophils* - They have a single nucleus which is dark blue in colour. Granules are also blue in colour. They are 10 microns in size and constitute 0.5 to 1% of all WBCs.

- **Platelets - They are small round shaped red stained bodies (size - 2 microns) without a nucleus. They often appear in clusters and are 2.5 to 4 lakhs in one cubic mm. of blood.**

COLOUR OF NUCLEUS, GRANULES OF WBC, RBC AND PLATELETS DESCRIBED ABOVE IS SEEN ONLY IN STAINED SLIDE

IN AN UNSTAINED BLOOD SMEAR
1. RBCs ARE OF PALE YELLOW/PINK COLOUR.
2. WBCs AND PLATELETS HAVE NO COLOUR



MALARIA PARASITES ARE OF FOUR TYPES

1. *Plasmodium falciparum.*
2. *Plasmodium vivax.*
3. *Plasmodium malariae.*
4. *Plasmodium ovale.*

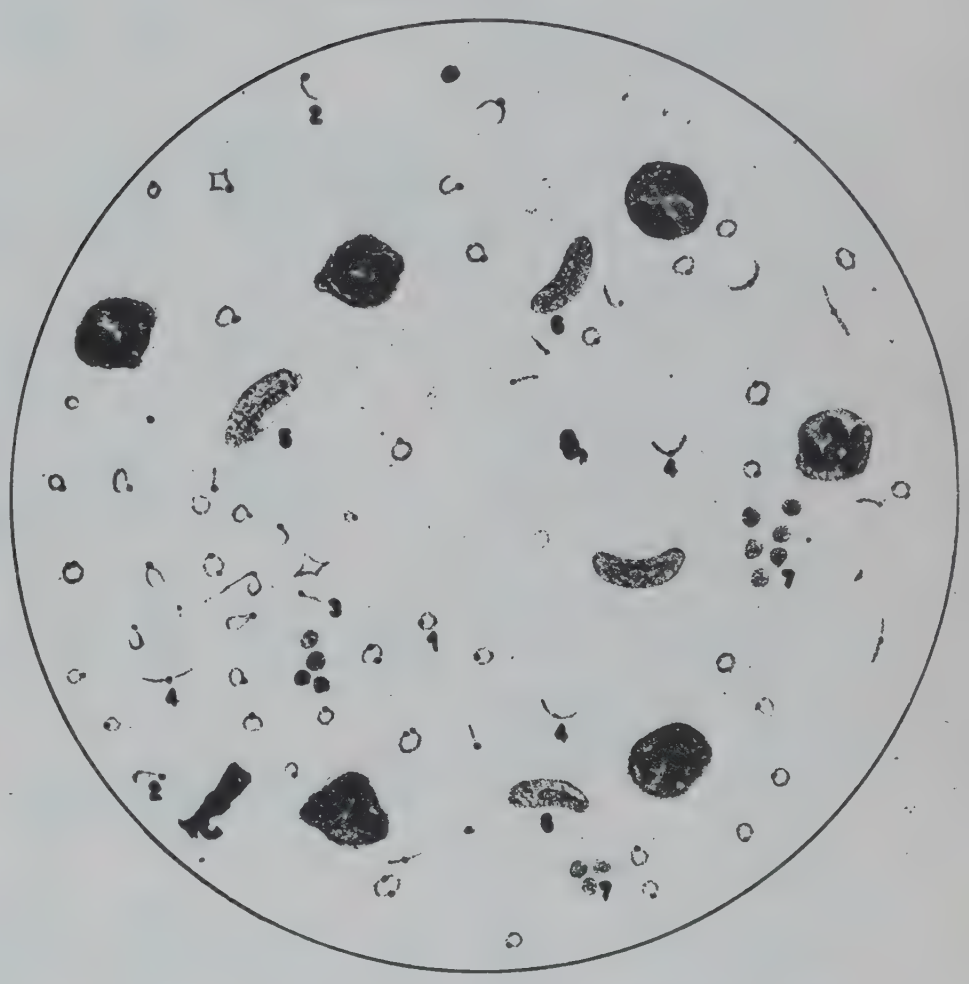
P. ovale is not found in India. Three species of the parasite are prevalent in India but *P. malariae* is very rare. It is commonly found in tribals, in forests and hilly areas. Other two species i.e. *P. vivax* and *P. falciparum* are found in every part of the country.

P. FALCIPARUM CAN CAUSE DEATH. IT IS VERY IMPORTANT TO HAVE AN EARLY AND CORRECT DIAGNOSIS AND TREATMENT OF ALL FEVER CASES SPECIALLY *P. FALCIPARUM* INFECTION.

Plasmodium vivax
THICK FILM SMEAR
(Diagrammatic)

1. RINGS. 2. EARLY TROPHOZOITES. 3. MATURE TROPHOZOITE. 4. EARLY SCHIZONTS. 5. SCHIZONT. 6. RUPTURED SCHIZONT 7. EARLY MALE GAMETOCYTE 8. MATURE MALE GAMETOCYTES 9. EARLY FEMALE GAMETOCYTE. 10. MATURE FEMALE GAMETOCYTE. 11. BLOOD PLATELETS.

Plasmodium falciparum
THICK FILM SMEAR
(Diagrammatic)

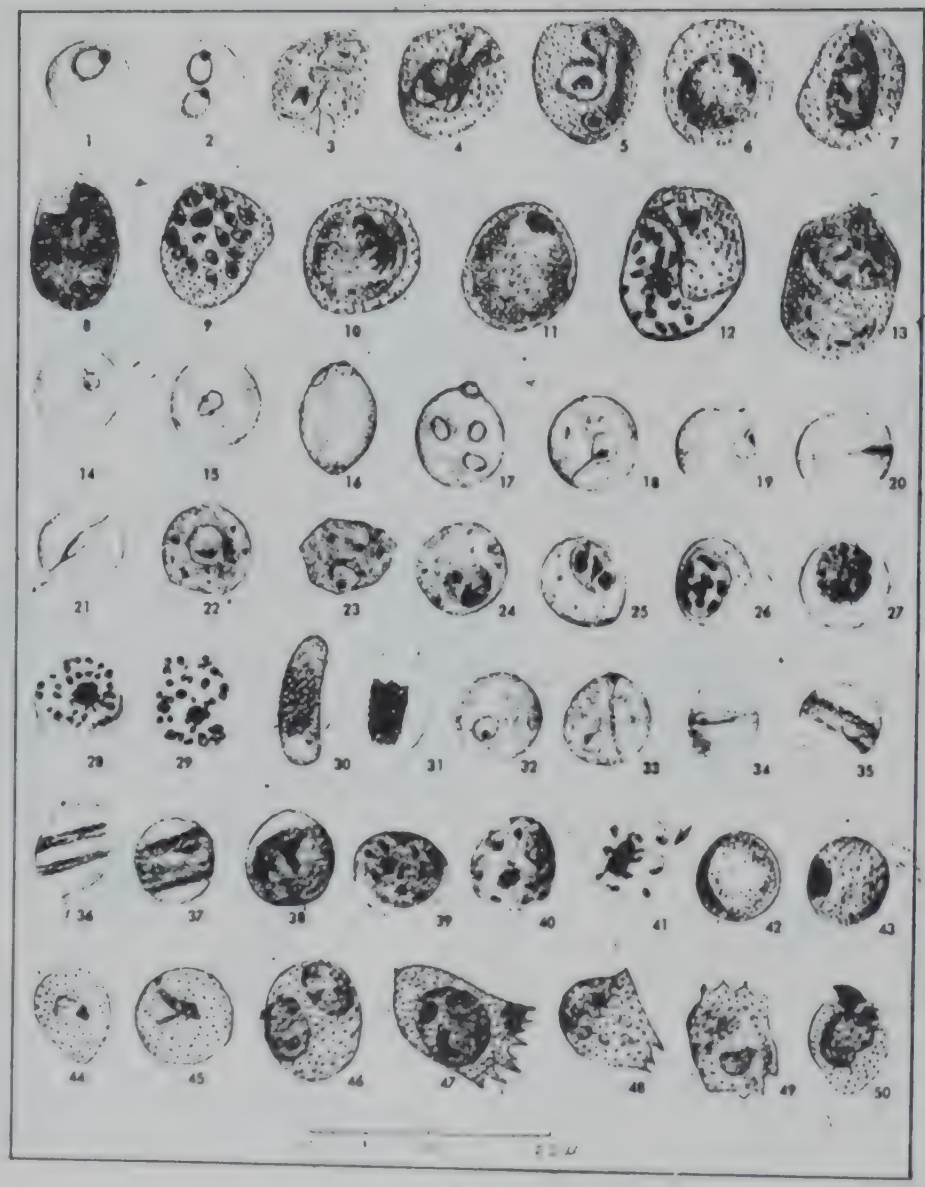


1-4 RINGS. 2. COMMAS. 3. EXCLAMATIONS. 4. WINGS. 5. MALE GAMETOCYTES. 6. FEMALE GAMETOCYTES. 7. BLOOD PLATELETS.

MALARIA PARASITES OF MAN

Plasmodium vivax. Figs. 1-13.
1, 2. Young trophozoite 'ring' forms. 3-5 Growing trophozoite forms. Parasites show marked amoeboid activity. Corpuscles are enlarged, pale and show Schuffner's dots. Granules of pigment are scattered throughout the cytoplasm in figs. 4 and 5.
5-8. Developing schizonts.
9. Mature Schizont. The pigment has collected into a loose mass, and formation of merozoites has taken place.
10. Mature microgametocyte. The chromatin is diffuse and the cytoplasm stains a pale blue colour. 11. Mature macrogametocyte. the cytoplasm stains deep blue. The chromatin is compact and stains deeply.
12. Schizont and gametocyte within the same corpuscle.
13. Two schizonts within the same corpuscles.

Plasmodium falciparum. Figs. 14-31
14. Young ring form.
15. Young ring form with double chromatin dots.
16, 17. Multiple infection of cropuscles, also showing marginal or accole forms.



18-21. Tenue forms corresponding to *P. tenuei.*
22-23. Large ring forms. The infected corpuscles show Maurer's dots.
24. Growing trophozoite from a culture.
The parasite has lost the vacuole and a jet-black compact mass of pigment has appeared.
25-27. Developing schizonts from a culture.
28-29. Mature schizonts from a culture.
30. Mature microgametocyte.
31. Mature macrogametocyte.

Plasmodium malariae. Figs.32-43
32. Young ring form.
33-36. Growing trophozoite forms showing characteristic band forms.
37. Large rectangular band form.
38. 39. Developing schizonts.
40.41. Mature schizonts.
42. Mature microgametocyte.
43. Mature macrogametocyte.

Plasmodium ovale. Figs. 44-50 (After James, 1933)
44, 45. Growing trophozoites.
46, 47. Developing schizonts.
48. Mature schizont.
49. Mature microgametocyte.
50. Mature macrogametocyte.

COMPARATIVE DESCRIPTION OF DIFFERENT STAGES OF *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN THICK FILM*

Stage of parasite	*P. falciparum*	*P. vivax*	Comparative description
1	2	3	4
Ring	Small rings with shining red chromatin dot, bluish violet coloured hair like small cytoplasm. The parasite also contains an empty space-vacuole. Sometimes 2 chromatin dots are present in a ring. In accordance with the characteristics of this species, the high number of rings are usually present in the smear. Hence, for identification, it is not always necessary that the crescents should be present. Diagnosis can be given on the physical appearance of the ring.	It is a large ring as compared to the ring of *P. falciparum* (nearly 2 to 2½ times bigger). Chromatin is red in colour but usually only one chromatin is present on each ring. Cytoplasm is regular, round and slightly thick. Vacuole is clearly visible. Ring stages are not present in large number.	It is difficult to identify the parasite species during ring stage but the following factors help in identification of *P. falciparum* rings; 1. Rings are in large number and no other stages are seen. 2. Some rings are with 2 chromatin dots. 3. Rings are smaller in size. 4. Sometimes rings are in the shape of comma, swallow or wings. Rings of *P. vivax* are big in size usually with only one chromatin. The other stages like trophozoites, schizonts and gametocytes are also seen along with rings in the same smear.

***QUALITY OF GOOD THICK FILM**

- It is 10 mm. away from the edge.
- It is round in shape with 10 mm. diameter.
- Its thickness contains 10 layers of red blood cells.
- 10 to 12 W.B.Cs. should be visible in one oil immersion field of microscope.

1	2	3	4
Growing Trophozoite	The rings is of bigger size, almost like a *P. vivax* ring. Cytoplasm is thick and regular but the size of ring is not too big. Vacuole is present.	Chromatin increases in size and cytoplasm becomes amoeboid in shape. Vacuole is visible. Yellowish brown pigments are seen all over the cytoplasm and Schuffner's dots which are fine pink/red in colour, numerous in number are also seen.	After proper staining, Schuffner's dots which are bright pink in colour are seen in large number in *P. vivax* species. These are not present in *P. falciparum*. In this species, Maurer's dots are seen if the staining is very good. This stage of parasite with Maurer's dots is found in very small number of *P. falciparum* blood slides.
Mature Trophozoite	As we take blood from peripheral circulation for making a blood smear it is not possible to see mature trophozoite stage. From this stage onwards, further development of parasite takes place in blood vessels of internal organs. The various changes or developmental phases in this species are similar to those of *P. vivax*.	In this stage, chromatin is found as a big collection of red coloured material at one place. Sometimes it simulates the chromatin in female gametocyte. Cytoplasm is amoeboid in shape. Occasionally, 2 vacuoles are seen. If the staining is good, by even Schuffner's dots are to be clearly seen. Pigment is found scattered all over the cytoplasm.	It is not necessary to have comparative description as this stage is not found or found rarely in the peripheral blood in case of *P. falciparum* infection.

1	2	3	4
Developing Schizont	This stage is not found in the peripheral circulation. Note - only on very rare occasions these stages are seen in peripheral blood.	The division of chromatin starts in this stage, so two or more than two chromatins are seen. Cytoplasm with a bluish violet hue also starts breaking up. Shuffner's dots are seen very clearly. Pigment may be seen alongwith parasite structure.	It is not necessary to have a comparative description as this stage is not found in peripheral circulation in the case of *P. falciparum* infection, and only peripheral blood is used for the preparation of a smear.
Mature Schizont	This stage is not found in the peripheral blood but they develop in the internal blood vessels Chromatin divides into 8 to 32 parts (average - 18). Each chromatin dot has got some portion of cytoplasm attached to it. Vacuoles are not seen. Maurer's dots and pigments are visible	In this stage of parasite chromatin gets divided into 12 to 24 parts (average - 16). Different parts of chromatin are seen as small dots but each of them has got a small portion of cytoplasm attached to them. Vacuole is not seen. Schuffner's dots are seen and cluster of yellowish brown pigment is also observed.	It is not necessary to have a comparative description as this stage is not usually found in blood slides in the case of *P. falciparum* infection. Only in very rare cases these are seen in blood smears.

1	2	3	4
Gametocyte	This is the sexual stage of parasite which is very distinctive and seen in the blood slide. Both male and female forms are present and it is difficult but not impossible to differentiate between the two. 1. In *P. falciparum* species gametocyte is shaped like a crescent or a banana. 2. In female gametocyte the chromatin is compact and deep red in colour. In male the chromatin is found scattered in the middle part of the parasite. 3. Female gametocyte is bigger in size as compared to male and the ends of the crescent are rounded in male while they are sharp in female. 4. Maurer's dots are seen in both male as well as female crescents.	This is the sexual stage of parasite and both male and female forms are present. 1. They are round in shape. 2. Chromatin is found compact and deep red in colour in female and it is found scattered in males. This chromatin is bigger than that of trophozoite. If this chromatin is seen at periphery, then this is a diagnostic point. 3. Female gametocyte is bigger than male. 4. Schuffner's dots are seen in male as well as female gametocytes but they are less in number as compared to trophozoite stage.	1. While comparing the gametocyte of *P. falciparum* and *P. vivax* their shape should be considered. 2. If stippling like Schuffner's dots or Maurer's dots are seen, then it is easy to differentiate. 3. Only good staining can help with the colour and arrangement of pigments and cytoplasm to differentiate the sex of gametocyte.

13704

1	2	3	4
Gametocyte	5. Pigments in female are found arranged round the chromatin but they are scattered all over the cytoplasm in male gametoycyte. 6. There is no vacuole in the crescent. 7. Cytoplasm which is crescent in shape is dark bluish violet in the case of female while the colour is lighter in the case of male.	5. Yellowish brown pigments are found arranged around the chromatin in female while they are scattered in males. 6. Vacuole is not seen. 7. Cytoplasm is round, has a bluish violet blut. Colour is faint in male and dark in female.	

It is very important that the cytoplasm, chromatin dots and the stippling in respect to their colour, shape and size are observed carefully for correct diagnosis of the species and stage of the parasite.

1. In the pages which follow, specimen outlines of different charts are given. In the charts maintained at the PHC laboratories, the same columns will be made for all months as indicated for the month of January as an example.

2. In the rows/columns of the chart, wherever necessary, dates, S.No., Code No. number of the slides, number of the Section, Sector, DDC, FTD, passive agency should be written.

JOB CHART OF LABORATORY TECHNICIAN

1. To record the particulars of all blood slides received in appropriate registers. To stain and examine them.
2. To inform daily about all slides found positive on examination to all concerned officers.
3. To complete daily dairy and get it signed by the Medical Officer in-charge.
4. To keep up-to-date information about antimalarial tablets and slides. To receive necessary reports from MPW and to supply clean glass slides (old and new) to them during periodic meetings held at PHC.
5. To fill up necessary graphs and charts regularly and display them in the Laboratory.
6. To send reports promptly at the specified time.
7. To send blood slides for cross-checks, as asked for, within 24 hours after receipt of the code number.
8. To inform Medical Officer in-charge of PHC and District Malaria Officer in case of increase in backlog and to follow their instructions.
9. To inform concerned authorities immediately if there is an abnormal increase in the number of positive cases.
10. To inform concerned Officer if for a long duration no slide is received from any particular village or section.

Registers :

1. To enter information about receipt and examination of blood slides in *MF 8.*
2. To enter information about positive slides and the treatment in *MF 7.*
3. To enter montly epidemiological information section-wise and village-wise in *MF 9.*
4. To maintain 'Malaria mobidity chart' showing the details of passive surveillance at the institution.
5. To maintain the 'Daily Examination Chart' and 'Thalati Chart'.

Reports :

1. To send weekly epidemiological report *(MF 11)* every Saturday to the Central Hq.
2. To send montly technical report in *MF 4-5.*
3. To send monthly passive agencies and FTD reports in *MF 10.*

TREATMENT OF FEVER/MALARIA POSITIVE CASES

Presumptive Treatment

A single dose comprising of 600 mg. (adult dose) of Chloroquine Phosphate (4 Aminoquinolines) is administered.

Age in Years	*Tablet Chloroquine Phosphate (150 mg. base)*	
	(mg. base)	*No of tablets*
< - 1	75	½
1 - 4	150	1
5 - 8	300	2
9 - 14	450	3
15 - >	600	4

Radical Treatment for *P. vivax, P. malariae* and mixed infections:

The adult dose/drug schedule is as follows:

A single dose of 600 mg Chloroquine (10 mg/kg. body weight) and 15 mg Primaquine (0.25 mg/kg. body weight) on the first day followed by 15 mg Primaquine (0.25 mg/kg. body weight) daily for the next four days.

Age in Years	*Tablet Chloroquine (150 mg. base)*		*Tablet Primaquine (2.5 mg. base)*	
	Single dose		*Daily dose for 5 days*	
	(mg. base)	*No. of tablets*	*(mg. base)*	*No. of tablets*
< - 1	75	½	nil	nil
1 - 4	150	1	2.5	1
5 - 8	300	2	5.0	2
9 - 14	450	3	10.0	4
15 - >	600	4	15.0	6

Radical Treatment for *P. falciparum*

The Adult dose is:

- 1500 mg Chloroquine in three divided daily doses (i.e. 600 mg each on 1st and 2nd day and 300 mg on 3rd day) plus 45 mg Primaquine on first day. This dose is suitably adjusted for other age groups.

CAUTION : INFANTS AND PREGNANT WOMEN ARE NOT TO BE GIVEN PRIMAQUINE

CHLOROQUINE PHOSPHATE

The toxicity is nil/minimal at the recommended dose under the National Malaria Eradication Programme.

TOXICITY: - Gastric irritation, nausea, vomiting, headache, pruritus, blurring of vision.

CHRONIC TOXICITY: **Prolonged use of large dosage of Chloroquine for weeks or months**

- Ocular damage like neuro-retinitis.
- Pigmentation of skin, nail-bed and palate.
- Neutropenia specially in long continued use.
- Skin rash.

REMEDY: **DRUG SHOULD NOT BE ADMINISTERED ON EMPTY STOMACH.**
The above symptoms usually disappear soon after discontinuation of the drug.

PRIMAQUINE

TOXICITY: At the recommended dosage, no symptoms of toxicity are likely to occur.

- Anorexia, nausea, vomiting, epigastric distress, abdominal pain and cramps.
- Hemolysis passage of dark urine, dyspnoea - (difficulty in breathing).
- Methaemoglobinaemia (manifested by cyanosis of nail-beds, lips, earlobes, tips of nose and tongue).

CHRONIC TOXICITY: In addition, there may be bone marrow depression after prolonged use or in cases sensitive to this drug.

- Anaemia, leukopenia.
- More severe toxic manifestations rarely occur in patients. The haemolytic action of Primaquine is related to the presence of certain hereditary enzyme deficiencies, particularly that of Glucose-6 - Phosphate Dehydrogenase (G-6-PD) - may lead to haemoglobinuria, renal failure.

CONTRA-INDICATION AND CAUTION :

- KNOWN G-6-PD DEFICIENT PATIENTS, INFANTS, PREGNANT WOMEN AND PATIENTS WITH TENDENCY OF GRANULOCYTOPENIA,
- PRIMAQUINE SHOULD NOT BE ADMINISTERED ON EMPTY STOMACH.

REMEDY :

- THE ABOVE MANIFESTATIONS DISAPPEAR WHEN THE DRUG IS WITHDRAWN OR STOPPED.
- Patient should be removed immediately to Primary Health Centre; if not controlled, transfer to referral Hospital.

POISONING

- Symptoms may be any one of the listed above.

TREATMENT

- WHETHER ACUTE OR CHRONIC - FOLINIC ACID AT A DOSE OF 10-20 mg. DAILY SHOULD BE GIVEN

PYRIMETHAMINE

TOXICITY

- At the recommended dosage the toxicity is very low. Long term administration of Pyrimethamine may result in

- Gastrointestinal disturbances, ulceration of mouth, loss of hair and anameia.

REMEDY

- WITHDRAWAL OF THE DRUG
- ADMINISTRATION OF FOLINIC ACID 10-15 mg. DAILY.

CAUTION

PYRIMETHAMINE ALONE IS CONTRAINDICATED. It should always be used in combination with sulphadoxine/sulfalene.

- It should be used only for *P. falciparum* cases which are resistant to chloroquine.

- Keep the drug out of reach of the children.
- **PYRIMETHAMINE SHOULD NOT BE ADMINISTERED ON EMPTY STOMACH.**

POISONING :

- Acute poisoning in children has been reported, who have consumed large number of tablets
- Acute poisoning may occur with 100 mg - 250 mg. of the drug.
- The symptoms are:

- Convulsions
- Loss of consciousness and collapse with fatal outcome.

TREATMENT

- GASTRIC LAVAGE, 5-10 mg. DIAZEPAM GIVEN SLOWLY BY INTRAVENOUS OR INTRA-MUSCULAR INJECTION, FOLINIC ACID 10-12 mg. DAILY.

Epidemilogical Parameters in Malaria Eradication Programme

1. Annual Blood Smear Examination Rate (ABER) Calculation formula

$$\frac{\text{Number of blood smears examined in a year}}{\text{Total population}} \times 100$$

Epidemiological significance

This parameter reflects the efficiency and adequacy of case detection mechanism. A minimum ABER of 10% per year has been fixed under the Malaria Eradication Programme based on estimates of fever rate in India. It has been laid down that the ABER should not be less than 1% per month during the transmission period.

2. Annual Parasite Incidence (API) Calculation formula

$$\frac{\text{Total No. of blood smears positive for malaria parasite in a year}}{\text{Total population}} \times 1000$$

Epidemiological Significance

This parameter depends upon the adequacy of case detection mechanism i.e., ABER. If ABER is adequate, this parameter is the most important criterion to assess the progress of eradication programme.

At present this parameter i.e., API is used under the MPO for determining the areas to be brought under spray operations.

3. Annual falciparum Incidence (AfI) Calculation formula

$$\frac{\text{Total No. of blood smears positive for } \textit{P. falciparum} \text{ in a year}}{\text{Total population}} \times 1000$$

This is expressed as the proportion of total number of blood smears found positive for *P. falciparum* infection in the total population under malaria surveillance.

4. **Slide Positivity Rate (SPR) Calculation formula**

$$\frac{\text{Total No. of blood smears found positive for malaria parasite}}{\text{Total No. of blood smears examined}} \times 100$$

Epidemiological significance

This parameter is less dependent on case detection mechanism (ABER). Whenever the case detection mechanism is inadequate, this is a dependable parameter for determining the programme of containment measures and gives information of parasitic load in the community. It is more reliable than API even for areas where ABER fluctuates from year to year.

5. **Slide falciparum Rate (SfR) Calculation Formula**

$$\frac{\text{Total No. of blood smears found Positive for } \textit{P. falciparum}}{\text{Total No. of blood smears examined}} \times 100$$

Epidemiological significance

This parameter also depends less on the case detection mechanism in space and time. If such a mechanism is adequate, it pinpoints areas of ***P. falciparum*** preponderance and indicates the necessity for intensification of intervention measures on priority basis to control ***Plasmodium falciparum*** infection which is responsible for malaria mortality in India.

6. ***P. falciparum* Percentage (Pf %) Calculation Formula**

$$\frac{\text{Total No. of blood smears found positive for } \textit{P. falciparum}}{\text{Total No. of blood smears positive for malaria parasite}} \times 100$$

Epidemiological significance

This parameter gives the relative proportion of ***P. falciparum*** infection and identifies trends of ***P. falciparum*** incidence in relation to total case load of malaria parasite in the community.

Chart and Graphs

1. To prepare a master chart about active collection and positive blood slides for every sector.

2. To prepare a master chart about passive agencies with number of fever cases treated, blood slides collected and positive blood slides detected.

3. To prepare a backlog chart of blood slides which have not been examined.

4. To prepare a RT backlog chart in which number of cases given RT should be given as compared to total positive cases detected every month.

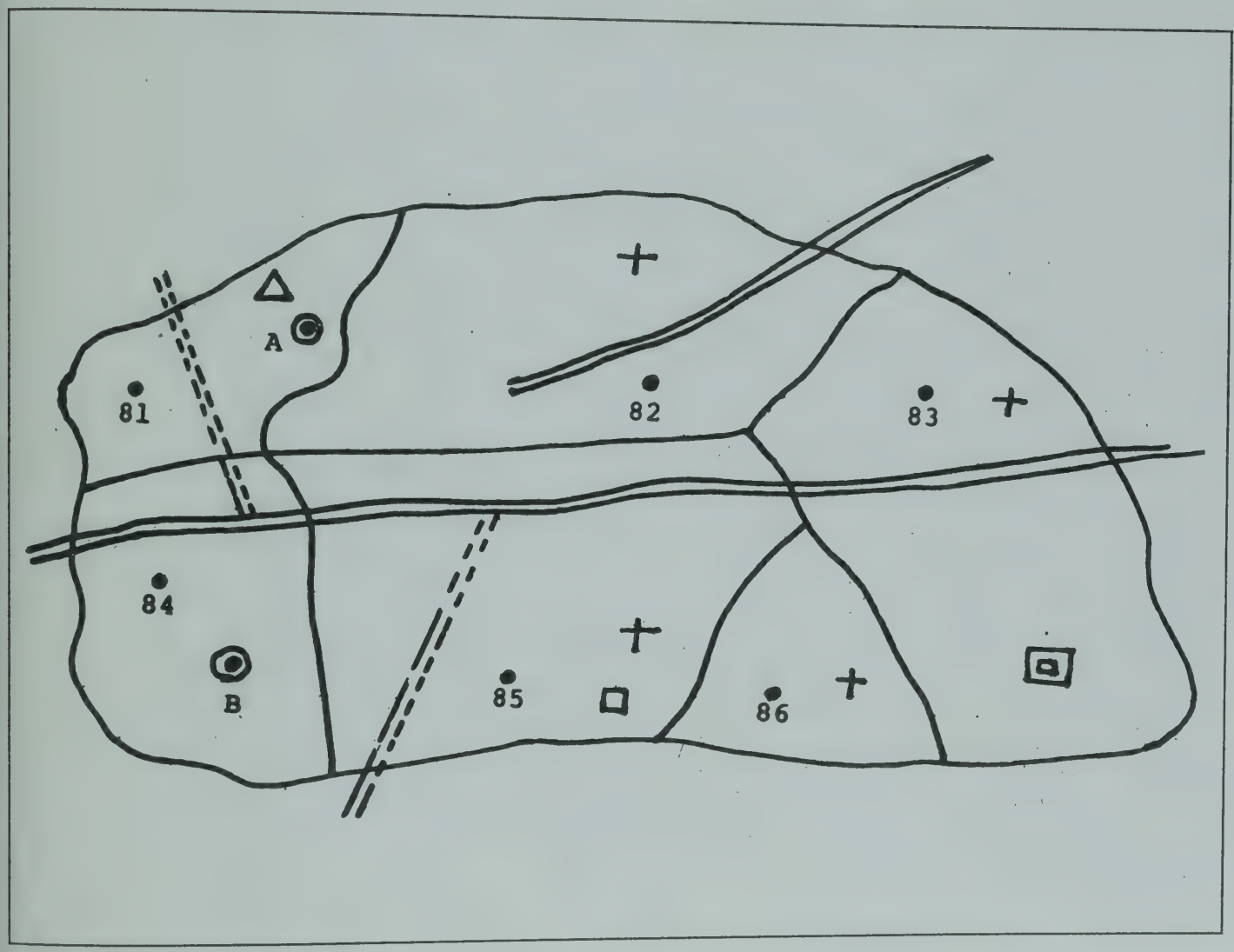
5. To prepare a graph showing montly blood slide collection and number of postive cases.

ALL NECESSARY CHARTS ARE GIVEN AT THE END OF THIS BOOKLET.
CHARTS SHOULD BE MADE AS PER COLUMNS INDICATED.

CHART No. 1

MAP OF PHC

POPULATION



Index

1. PHC
2. Sector
3. Section
4. Population
5. Area in Sq. Kms.
6. No. of Villages
7. No. of Paras (Hamlets)
8. No. of Human Dwellings
9. No. of Families
10. Sub-Centre
11. Mini PHC
12. Ayurvedic Dispensary
13. All-weather Dispensary
14. Fair-Weather Roads
15. Rivers/Streams

MF - 1

Sl. No.	House No.	Name of Head of Household	Names of other Members	Age	Sex	Remarks

MF - 2

FOR REPORTING OF BLOOD SMEARS BY MULTIPURPOSE WORKER/PASSIVE AGENCY

Name of Sub Centre ..... Name of the PHC .....

Code Number : .....

Sl. No.	Village	No. of house	Name of Head of Family	Name of patient/ person	Age & Sex	Sl. No. of blood smear	No. of chloroquine tablets given	Date of collection	Result	Remarks

Note : This proforma should be in triplicate and three copies forwarded to PHC Laboratory Technician, who will retain one copy and send the other two to MPW/Senior Health Inspector/Malaria Inspector.

Signature of Microscopist Date of examination by the Microscopist Signature of MPW/passive agency

MF - 4

Primary Health Centre ..... Taluk .....

Sub Centre	Active B.S.			Mass & Contact B.S			Passive B.S.			Total Blood Smears			P.v. Incidence				P.f. Incidence				Detail of the parasite					Radical Treatment		
	Collected	Examined	Positive	Collected	Examined	Positive	Collected	Examined	Positive	Collected	Examined	Positive	0-1 Year	1-4 Year	5-14 Year	> 15 Year	0-1 Year	1-4 Year	5-14 Year	5-14 Year	P.V.	P.f (R)	P.f. (Rg)	Mix	Total	P.V.	P.f.	Total

MF - 6

CHART SHOWING THE BLOOD SMEARS COLLECTED FROM ACTIVE & PASSIVE AGENCIES AT PHC

..... FOR THE YEAR 1999

ACTIVE

Name of the Sub-Centre with S.C. No.	January					February					March					April					May					June					July					August					September					October					November					December					Remarks
	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	B/S Collected	Pv	Pf	Mix	Total	
TOTAL																																																						

PASSIVE

Name of the DISP & FTD																																																								

MF - 7

POSITIVE REGISTER

Sl. No.	Name of Patient	Age	Sex	Date of B.S. Collection	Date of B.S. Examination	Result	Date of Intimation	RT		Follow up Smear		Remarks
								From	To	Date	Result	

MF - 8

Register of blood smears received and examined
(Subcentre - wise)

Name of Subcentre ..... PHC .....

Sl. No	Date of Receipt	Section No./ Hosp	Total smears received	Blood Smears Number		Date of collection		Date of Examn.	Remarks
				From	To	From	To		

MF - 9

EPIDEMIOLOGICAL EVALUATION MASTER REGISTER
(SUBCENTRE-WISE, VILLAGE - WISE & MONTH - WISE)

..... Month ..... Primary Health Centre

Sub Centre Village	Name of the Male Health Assistant	First Fortnight													Second Fortnight												Total for the Month
		1	2	3	4	5	6	7	8	9	10	11	12	Tota	1	2	3	4	5	6	7	8	9	10	11	12	Total	

MF - 11

WEEKLY SAVINGRAM REPORTS UNDER NATIONAL MALARIA ERADICATION PROGRAMME

..... Week report ..... Primary Health Centre ..... Taluk, From Dt..... To dt.....

Subcentre No.	Subcentre	Blood Smears					During the week				From 1-1-98						Detail of Balance					Sent Outside				Received from Outside			
							M.P. cases		R.T. Given		M.P. cases		R.T. Given		Balance									R.T. Given				R.T. Given	
		O.B.	Collected	Examined	Sent outside	Balance	Total	P.f.	Total	P.f.	Total	P.f.	Total	P.f.	Total	P.f.	Child	Pregnant	Not	Under	Sent out	Total	P.f.	Total	P.f.	Total	P.f.	Total	P.f.

Signature of the medical officer

Daily Examination Chart at PHC Laboratory

Date	Opening Balance					Receipt					TOTAL					Exd. by Lab Tech					Exd. by HQ Jr. HI					Progressive TOTAL	Closing Balance					Positive				Remarks
	Active	Passive	M&C	F-UP	TOTAL	Active	Passive	M&C	F-UP	TOTAL	Active	Passive	M&C	F-UP	TOTAL	Active	Passive	M&C	F-UP	TOTAL	Active	Passive	M&C	F-UP	TOTAL		Active	Passive	M&C	F-UP	TOTAL	Pv	Pf.	Mix	Total	

Malaria Morbidity Chart

Sl. No.	Name of th Month	Total OPD	New OPD	Fever Cases	B.S. drawn	Chloriquine tabs given	Result	RT	Remarks

Thalati Chart (Ladder Chart) Showing the period of Examination of blood smears for the year .....

Name of the PHC :

Population : Taluk : District :

Month	Total Blood smears received	No. of Blood smears examined / M.P.P. Cases												Diversion	Total	MPP	P.f.
		January	February	March	April	May	June	July	August	September	October	November	December				
JANUARY																	
FEBRUARY																	
MARCH																	
APRIL																	
MAY																	
JUNE																	
JULY																	
AUGUST																	
SEPTEMBER																	
OCTOBER																	
NOVEMBER																	
DECEMBER																	
GRAND TOTAL																	

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